

The case for an autoimmune aetiology of type 1 diabetes

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Summary

Type 1 diabetes (T1D) develops when there are insufficient insulin-producing beta cells to maintain glucose homeostasis. The prevailing view has been that T1D is caused by immune-mediated destruction of the pancreatic beta cells. However, several recent papers have challenged the long-standing paradigm describing T1D as a tissue-specific autoimmune disease. These authors have highlighted the gaps in our knowledge and understanding of the aetiology of T1D in humans. Here we review the evidence and argue the case for the autoimmune basis of human T1D. In particular, recent analysis of human islet-infiltrating T cells brings important new evidence to this question. Further data in support of the autoimmune basis of T1D from many fields, including genetics, experimental therapies and immunology, is discussed. Finally, we highlight some of the persistent questions relating to the pathogenesis of human type 1 diabetes that remain to be answered.

Keywords: type 1 diabetes, aetiology, HLA, autoantibody, T cells

Introduction

Type 1 diabetes (T1D) is caused by a deficiency in insulin production resulting from the destruction of the pancreatic beta cells. Evidence supporting an immunological basis of beta-cell destruction in type 1 diabetes has accumulated over the past 50 years [1]. This has included the histopathological appearance of insulinitis, the discovery of islet-cell autoantibodies and the genetic association with the human leucocyte antigen (HLA) locus and subsequently with many other loci that encode immune genes. In addition, some telling 'experiments of nature' have been described. These include recurrence of diabetes in identical twin segmental pancreas transplants and transfer of diabetes with bone marrow transplantation. However, the paradigm that beta-cell destruction is caused by an autoimmune response directed against the insulin-secreting beta cells has been challenged recently [2–4] (See Box 1). This challenge is encouraged by the field's failure to convert knowledge of autoimmunity in T1D to effective therapies for prevention, or reversal, in patients. Furthermore, for logistical, technical and ethical reasons it has been very difficult to collect evidence in humans as compelling as the evidence gained from studying in animal models. These challenges against

the immune-mediated paradigm of human T1D are, to an extent, deliberately provocative, with the worthy goal of stimulating discussion. However, they also plant the seed in the minds of scientists and reviewers that the autoimmune response in T1D is secondary to tissue damage and unrelated to disease pathogenesis. This is a major departure from current thinking that challenges decades of work. Recently, work by several groups [5,6], including our own [7], has produced novel data that we believe tip the balance in support of an autoimmune basis of human T1D (summarized in Fig. 1); therefore, a clear analysis of this proposition is needed. In this review we examine, and seek to reaffirm, the case for the autoimmune pathogenesis of human T1D.

Beta-cell antigen-specific T cells infiltrate the islets in T1D

Immune cells infiltrate the islets in T1D. Infiltration of the pancreatic islets of Langerhans by immune cells was first reported by Schmidt in 1902 [8]. Immune-cell infiltration was termed 'insulinitis' by the Swiss pathologist von Meyenburg [9]. In 1965 Gepts [10] reported that 15 of 22 (68%) recent-onset T1D cases had insulinitis. This conclusion was

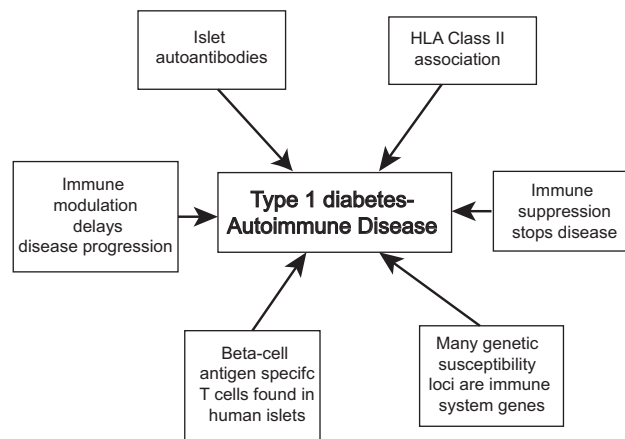


Fig. 1. Summary of evidence for the autoimmune aetiology of human type 1 diabetes.

supported by Foulis *et al.* [11], who reported that 47 of 60 (78%) people with T1D who died before they were 20 had insulinitis. Analysis of islet-infiltrating cells in recent onset T1D samples revealed that T cells, B cells and macrophages account for most of the infiltrating cells [12]. However, progress in this area has been slow because, as pointed out by In't Veld [13], until 2010 only 151 pancreas samples were available for histological analysis from individuals who died with T1D.

The mild immune cell-infiltration in the pancreatic islets of organ donors who suffered from type 1 diabetes has been cited as evidence that type 1 diabetes is not caused by an autoimmune response [2,4]. Currently, the histological diagnosis of type 1 diabetes requires that ≥ 15 CD45⁺ be detected in more than three islets [14]. Compared to the non-obese diabetic (NOD) mouse, this is a very modest degree of infiltration [15,16]. However, current histological techniques can examine only a very small proportion of the pancreas. Studies using samples from nPOD (Network for Pancreatic Organ Donors with Diabetes) have revealed that infiltration is not homogeneous, but that some parts of the pancreas have more infiltration than others [17]. Histological examination of human tissues can only be performed at one point in time and does not provide any insight into the dynamics of immune cell infiltration. Furthermore, T1D can develop over many years, or decades, which makes a slow, chronic, immune cell infiltration and beta cell destruction a plausible pathogenic mechanism in humans.

CD4⁺ T cells, specific for beta-cell antigens, are present in the islets of individuals who suffered from T1D [5,7]. We cloned CD4⁺ T cells from the residual pancreatic islets of an organ donor who suffered from T1D. This donor had the highest T1D risk genotype: HLA-DR3-DQ2; HLA-DR4-DQ8, and was homozygous for the type 1 insulin variable number tandem repeat (VNTR). Analysis of CD4⁺ T cell clones revealed that ~26% (14 of 54 clones) recognized epitopes derived the C-peptide of proinsulin [7].

Remarkably, all the clones were restricted by HLA-DQ8 or the HLA-DQ8 transdimer [7]. This work provides new evidence supporting the pathogenic role of human CD4⁺, proinsulin-specific T cells in human T1D.

Beta-cell antigen-specific CD8⁺ T cells have also been documented in the pancreatic islets from deceased organ donors who suffered from T1D. Coppetiers *et al.* [5] used HLA-A2 tetramers incorporating beta-cell antigen-derived peptides to stain a series of pancreatic sections from individuals who suffered from T1D. HLA-A2/peptide epitope staining of sections from HLA-A2⁺ T1D cases revealed infiltration by CD8⁺ T cells in eight of 10 cases. CD8⁺ T cells specific for epitopes from insulin, islet-glucose-6-phosphatase catalytic subunit-related protein (IGRP), insulinoma antigen 2 (IA-2), glutamic acid decarboxylase-65 (GAD-65) and pre-pro islet amyloid protein (ppIAP) were detected. The T cells stained by these tetramers have been shown to kill beta cells, suggesting that CD8⁺ T cells specific for these epitopes may be pathogenic [18–20]. This work revealed clearly, for the first time, the presence of HLA-A2-restricted CD8⁺ T cells specific for epitopes from beta-cell antigens. Interestingly, seven of the eight cases with detectable antigen-specific T cells had HLA-A2/insulin-specific CD8⁺ T cells within their islets.

A recent report of a NOD mouse study [21] has challenged the view that the pancreatic islets are infiltrated by pathogenic, beta-cell-specific T cells. This work suggests that NOD islets are infiltrated readily by immune cells. However, this study does not examine the egress of immune cells from the islets, nor the why their findings drew the opposite conclusion from earlier studies [22,23].

Genome wide association studies implicate many immune-related genes

Genetic studies have been powerful tools for dissecting the pathogenesis of human T1D [24,25]. During the past three

decades, genome wide association studies (GWAS) have established a list of loci associated with risk of T1D (reviewed by [26–28]). The great strength of GWAS studies is that they make relatively few assumptions; the goal is simply to determine which alleles are over- or under-represented in those who develop T1D.

We have known since before the 'GWAS era' that the HLA class II region has the strongest impact on T1D risk [29]. HLA molecules have highly polymorphic peptide-binding grooves that underpin the molecular specificity of the T cell responses [30]. The T cell receptor for antigen recognizes a composite shape formed by both the HLA molecule and the antigen-derived peptides it binds [31]. HLA-DQ2 (DQA1*05:01, DQB1*02:01) and DQ8 (DQA1*03:01, DQB1*03:02) confer the greatest risk of developing T1D [32]. Remarkably, individuals heterozygous for HLA-DQ2 and HLA-DQ8 are at greater risk of developing T1D than those with either HLA-DQ2 or -DQ8 alone [33]. The basis of this observation is unclear; however, HLA-DQ2; DQ8 heterozygous antigen-presenting cells (APC) can form two extra functional HLA molecules: a DQ8 transdimer composed of the HLA-DQ2 α chain paired with the HLA-DQ8 β chain (DQA1*05:01; DQB1*03:02), and, similarly, an HLA-DQ2 transdimer where the DQ8 β pairs with DQ2 α (DQA1*03:01; DQB1*02:01) [34]. These transdimers may promote beta-cell autoimmunity by presenting unique diabetogenic epitopes, or the high density of T1D-promoting HLA molecules (HLA-DQ2, -DQ8, -DQ2trans and -DQ8trans) may promote autoimmune CD4⁺ T cell responses against beta-cell antigens [34,35]. In contrast, some HLA alleles dominantly protect against T1D, HLA-DQB1*06:02, for example [36]. Hence, HLA class II alleles can both promote and protect from T1D [37].

The function of the HLA class II region is to present antigen-derived peptides to T cells. This allows HLA class II molecules to shape the T cell repertoire during T cell development in the thymus and to activate T cells in the draining lymph nodes. However, the reason why the HLA class II region is associated so closely with T1D, and other autoimmune diseases such as coeliac disease [31], is incompletely understood [30]. One plausible connection is that the association is due to how beta-cell derived epitopes bind to HLA molecules, such as HLA-DQ8. This may impact upon the escape of low-affinity T cells from the thymus and their subsequent activation by epitopes derived from beta cell antigens that are present at high concentration in the pancreas and its draining lymph nodes [38]. However, while there is accumulating evidence for this model in NOD mouse models, it remains unclear if a similar mechanism(s) operate in humans who develop T1D.

After the HLA class II region, genetic association studies have revealed that polymorphisms in the insulin gene have the second strongest association with T1D. This

locus maps to VNTR upstream of the insulin gene [39,40]. This polymorphism is believed to modulate proinsulin expression in the thymus, affecting central tolerance [39–41]. The strong association with the HLA-DQ locus, especially DQ8 and DQ2, and the association with the proinsulin locus leads to the prediction that proinsulin-specific CD4⁺ T cells restricted by these HLA-DQ alleles should be present in many people with T1D and play a pathogenic role. However, T cells such as this have proved difficult to find. They are, like the Higgs boson, an essential prediction of the autoimmune theory that has, until recently, not been found. Although proinsulin-specific CD4⁺ T cells have been found in peripheral blood and the pancreatic lymph nodes, they have been restricted by other HLA molecules such as HLA-DR4 [6,42]. Nonetheless, the genetic association studies strongly implicate HLA-DQ2- and DQ8-restricted CD4⁺ T cell responses in the pathogenesis of human T1D. In support of this notion, Durinovic-Bello *et al.* have shown that CD4⁺ T cells specific for insulin epitopes are more prevalent in the blood of subjects who express the high T1D risk, type 1 insulin VNTR [41].

More than 40 non-HLA genetic loci have been identified that impact upon the risk of developing T1D [25,43]. Many of these genes are associated with immune function. Examples include interleukin (IL)-2R α , protein tyrosine phosphatase non-receptor type 22 (PTN22), IL-10, chemokine receptor type 5 (CCR5) and IL-2 (reviewed by [44]). Many of the remaining genes are expressed the pancreatic islets, including SH2B adaptor protein 3 (SH2B3), Receptor tyrosine-protein kinase erbB-3 (ERBB3), PTN2 and cathepsin H (CTSH) [44,45]. Collectively, genetic studies have implicated polymorphisms in HLA class II, insulin and many other genes that regulate immune responses in the development of T1D in humans.

Autoantibodies specific for beta-cell proteins precede the onset of T1D

Some of the earliest evidence of the autoimmune aetiology of T1D came from the discovery that antibodies specific for components of the islets of Langerhans were present in the serum of people with T1D, but not healthy individuals. Islet cell-specific autoantibodies (ICA) were first reported in 1974 [46,47]. Many of the target antigens recognized by these antibodies have been identified since then. Insulin-specific antibodies were the first to be detected in the serum of individuals at risk of T1D [48]. Autoantibodies specific for several antigens have been identified, including GAD-65 [49], IA-2 [50] and zinc transporter 8 (ZnT8) [51]. Because of their specificity and sensitivity, islet autoantibodies have been extremely useful for identifying individuals who go on to develop T1D [52]. A simple relationship between the number of antigens recognized by islet autoantibodies has emerged – the greater the number

of beta-cell antigen-specific antibodies present in an individual's serum, the greater the risk of developing T1D [53–57]. Recently a large, international, multi-centred, longitudinal study analysed the relationship between islet autoantibodies on the risk of T1D. Newborns who had a first-degree relative with T1D and/or expressed a high-T1D risk HLA type were recruited and their islet antibodies measured regularly. They found that the risk of developing T1D by age 15 years increased with each additional antibody. By the age of 15, 0.4% of children without detectable islet antibodies [insulin autoantibodies (IAA), IA-2, GAD-65] had developed T1D, whereas 12.7% with a single islet autoantibody, 61.6% with two antibodies and 79.1% for children with three autoantibodies developed T1D by 15 years of age [57].

Beta-cell antigen-specific autoantibodies are not believed to be directly pathogenic. Islet autoantibodies do not have a direct cytotoxic effect on human islets [58], and transfer of maternal autoantibodies to the fetus does not increase the offspring's risk of T1D [59]. A single case of a 14-year-old boy with X-linked agammaglobulinaemia who developed T1D suggests that antibodies are not essential for T1D to develop in humans [60]. However, evidence from NOD mouse studies suggests that beta cell antigen-specific B cells and/or antibodies may facilitate the presentation of beta-cell antigens to pathogenic T cells, thereby promoting the development of T1D [61].

It is clear from many studies that antibodies specific for beta-cell antigens arise well before the onset of clinical T1D. For example, in the study described above, the median age of seroconversion was 2.1 years [57]. This supports a causal relationship between autoantibodies and autoimmune responses, in this case autoantibodies specific for beta-cell antigens, and T1D. However, it is not possible in human studies to analyse directly the causal relationship between the presence of autoantibodies and beta-cell destruction. Nevertheless, the further back in the natural history that autoantibodies and other evidence of autoimmunity are present the more consistent are the findings with the idea that autoimmunity is pathogenic. If these phenomena only occurred late in the disease course, for example after beta-cell mass has decreased, they would be more likely to be epiphenomena. Additionally, the dose relationship is also consistent with this – if more autoantibodies detected were not associated with more progression the link would be less compelling. However, autoantibodies are unlikely to provide proof of the autoimmune hypothesis on their own, because the evidence suggests that they are not pathogenic.

Detecting T cell responses to beta-cell antigens in peripheral blood mononuclear cells

The analysis of human T cell responses to beta-cell antigens has been challenging [62,63]. This work has been hampered

by two inter-related problems: first, the beta-cell-specific T cells are extremely rare in the peripheral blood; secondly, the antigen specificity of these cells is largely unknown. These problems are inter-related, because uncertainty around the identity of the target antigen/epitope undermines efforts to develop assays to detect T cell responses. In other words, the reason a response to a particular antigen/epitope was not detected could be that the antigen/epitope is irrelevant, or the assay is not sufficiently sensitive. Nonetheless, several sensitive assays for detecting autoantigen-specific T cells in human peripheral blood have been developed, including enzyme-linked immunospot (ELISPOT), carboxyfluorescein succinimidyl ester (CFSE)-based proliferation assays [64,65] and tetramer staining [66] (reviewed by [63]). Analysis of T cells that infiltrate the islets of individuals who suffered from T1D perhaps break this deadlock, because putative pathogenic, beta-cell antigen-specific T cells would be expected to accumulate there [67].

Many beta-cell proteins have been reported to be the targets of T cell responses implicated in human T1D (reviewed by [68,69]). The first antigens to be tested were those already shown to be the targets of autoantibody responses. Many epitopes derived from these antigens have been reported to be targets of T1D-associated T cell responses [68,69] but, because of the technical challenges in identifying antigen-specific T cells, and the variability inherent in human populations, no single epitope has emerged as pivotal to the pathogenesis of T1D in humans (reviewed by [68,69]). This does not, of course, argue that T1D is mediated by a 'non-autoimmune' mechanism. Rather, it suggests that new approaches are required to dissect the human autoimmune T cell responses.

Immune suppression reverses T1D

Further evidence for the autoimmune basis comes from immune-modulating therapies that have been tested in clinical trials for the treatment of T1D. The rationale for these studies is that if T1D is caused by an autoimmune T cell response directed against the pancreatic beta cells, interventions that inhibit T cell responses will halt disease progression. Many of the early efforts in the area have not led to effective therapies for T1D because of unacceptable toxicity profiles. Nevertheless, these studies provide unique evidence for an autoimmune aetiology of human T1D.

Treatment with the immunosuppressive drug cyclosporin leads to T1D remission. A series of trials conducted in the early 1980s showed that cyclosporin therapy led to T1D remission [70–72]. Interestingly, clinical benefit was seen only when the patients were treated within months of the onset of T1D. Residual beta cells are known to be present at the time of diagnosis [73]; in the absence of the ongoing autoimmune responses these cells are able to produce

sufficient insulin to restore glucose homeostasis. However, when cyclosporin therapy was ceased, T1D relapsed rapidly [74,75]. Similar findings were made in trials of prednisone and azathioprine. Treatment with these drugs led to improvements in glycated haemoglobin (HbA1c), insulin requirement and serum C-peptide in 50% of the treated subjects [76]. In contrast, 15% of the control group showed similar improvement [76]. Collectively, these trials revealed that immune suppression could be effective in treating patients with T1D. The toxicities associated with cyclosporin, prednisone and azathioprine preclude their use to treat T1D. Nonetheless, these trials raise the possibility that safer, more targeted immune therapies may be effective therapies for T1D.

Modulation of T cell function by anti-CD3 monoclonal antibodies (mAbs) delays the progression of T1D [77]. This approach to treating T1D emerged from NOD mouse studies showing that diabetes could be prevented in NOD mice treated with anti-CD3 [78,79]. Two humanized Fc-modified anti-CD3 monoclonal antibodies, teplizumab [80] and oteplizumab [81], have been generated and tested in clinical trials. Treatment with both anti-CD3 mAbs resulted in transitory improvements in serum C-peptide concentrations in subjects with recent-onset T1D [82–84] (reviewed by [85]). Whether or not anti-CD3 mAb therapy, in its current form, is suitable for widespread clinical use, it clearly delays the progression of T1D. Given the exclusive expression of CD3 by T cells, and the known impact of these antibodies on T cell function, these findings add support to the argument that T cells play a direct pathogenic role in human T1D.

Antibody-mediated B cell depletion with rituximab delays the progression of T1D in recently diagnosed individuals [86]. A placebo-controlled trial showed that subjects treated with rituximab had significantly lower HbA1c, insulin requirements and higher stimulated C-peptide levels than subjects who received the placebo [86]. Similar outcomes were reported in a trial of abatacept, a CTLA-4 (cytotoxic T lymphocyte antigen) -Ig (immunoglobulin) fusion protein that inhibits CD80/CD86 interaction with CTLA-4 [87].

Collectively, the trials reviewed here all indicate that modulation, suppression or adaptive immune function prevents or delays the progression of T1D. However, while all these agents delay the progression of T1D, none of them lead to an enduring remission. These findings are consistent with the immune-mediated pathology of T1D in humans and underscore the need for studies to investigate the mechanism(s) of relapse. In a review of cyclosporin trials published in 1993, Mahon *et al.* [88] conclude: 'The most important conclusion from the experience with cyclosporin therapy for IDDM is that an immune-mediated process causes beta-cell loss in human IDDM' [88].

Concluding comments

In summary, the notion that T1D is caused by an autoimmune T cell response against the insulin-producing beta cells is supported by genetic evidence, histology, intervention trials and *in-vitro* analysis of T cells. There are many gaps in our knowledge, including the nature of the initial event that triggers the autoimmune response that leads to T1D. Nonetheless, discarding the autoimmune model of T1D is, in our view, not supported by the evidence. Importantly, an alternative model of the aetiology of T1D must account for the observations that have already been confirmed by multiple studies.

The NOD mouse model has created a 'line in the sand' to which human studies have been compared. The severity of the autoimmune T cell response in the NOD mouse has created the expectation that the human autoimmune T cell response in T1D patients should be equally dramatic and consistent. This is clearly not the case. While acknowledging the many challenges associated with studying T1D in humans we argue that the evidence, accumulated over decades, supports the view that T1D in humans, like the NOD mouse, is caused by autoimmune T cell responses against the pancreatic beta cells. If our goal, as a community of clinicians/researchers, is to dissect the pathogenesis of human T1D and develop safe and effective new therapies we must grapple with the challenges of human-based research. We must also acknowledge the differences between animal models and humans: both their strengths and weaknesses. Most importantly, we must not let the 'absence of evidence for an autoimmune aetiology of T1D' be mistaken for the 'evidence that autoimmunity is absent' in people who develop T1D.

Future directions

Currently, the field's primary challenge is to develop models to directly assess the pathogenicity of human T cells. Much of the controversy stems from the paucity of evidence linking an immune response to the development of T1D. This will require increasingly sophisticated animal models that incorporate the relevant human cells, or molecules, to allow the direct analysis of pathogenesis. Studies in humanized animal models must be complemented and supported by the intensive analysis of human pancreatic tissues. The recent nPOD initiative [89,90] is an important step towards achieving this goal. Finally, future research should continue to scrutinize the hypothesis that T1D in humans stems from T cell-mediated destruction of the insulin-producing beta cells. However, while important gaps in our knowledge remain, we predict that the autoimmune paradigm of T1D will continue to serve the field well.

Box 1. Other models of the pathogenesis of type 1 diabetes

If T1D is not caused by an autoimmune responses how are the pancreatic insulin-secreting beta cells destroyed?

Korsgren and colleagues [91] have proposed that bacteria initiate an innate immune response that causes beta-cell destruction.

However, this model does not explain adequately the following observations:

1. Strong association with HLA-DQ2/DQ8.
2. Genetic linkage to *INS* and other immune system genes (*PTPN2*, *IL2*, etc.)
3. Specificity for beta cells.
4. Early age of onset.
5. Presence of beta-cell antigen-specific T cells in islets.
6. Impact of immune-suppressive therapies on T1D.

Disclosure

The authors have no disclosures to declare.

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